REMARKS

Sequence Listing:

The specification has been amended to include a sequence listing for the sequences found in the specification. A sequence listing paper copy has been submitted with this Amendment as additional sheets to the specification to be inserted before the claims however there are no sheets to be replaced. The sheets do not include new matter. A computer readable form has also been submitted and it is the same as the paper copy that has been added.

Respectfully submitted,

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EXAMPLES

The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present invention and are not to be construed as limiting the scope thereof.

EXAMPLE 1

Inhibition of luciferase gene expression by siRNA in liver cells *in vivo*.

A. Preparation of siRNA

Single-stranded, gene-specific sense and antisense RNA oligomers with overhanging 3' deoxynucleotides are prepared and purified by PAGE. The two oligomers, 40μM each, are annealed in 250μl of buffer containing 50mM Tris-HCl, pH 8.0 and 100mM NaCl, by heating to 94^0C for 2 minutes, cooling to 90^0C for 1 minute, then cooling to 20^0C at a rate of 1^0C per minute. The resulting siRNA is stored at -20^0C prior to use.

The sense oligomer with identity to the luc+ gene has the sequence:
5'-rCrUrUrArCrGrCrUrGrArGrUrArCrUrUrCrGrATT-3' (SEQ ID NO: 1)
and corresponds to positions155-173 of the luc+ reading frame. The letter "r" preceding a nucleotide indicates that nucleotide is a ribonucleotide.

The antisense oligomer with identity to the luc+ gene has the sequence:
5'-rUrCrGrArArGrUrArCrUrCrArGrCrGrUrArArGTT-3' (SEQ ID NO: 2)
and corresponds to positions155-173 of the luc+ reading frame in the antisense direction. The letter "r" preceding a nucleotide indicates that nucleotide is a ribonucleotide.

The annealed oligomers containing luc+ coding sequence are referred to as siRNA-luc+.

The sense oligomer with identity to the ColE1replication origin of bacterial plasmids has the sequence:

5'-rGrCrGrArUrArArGrUrCrGrUrGrUrCrUrUrArCTT-3' (SEQ ID NO: 3)
The letter "r" preceding a nucleotide indicates that nucleotide is a ribonucleotide.

The antisense oligomer with identity to the ColE1 origin of bacterial plasmids has the sequence:
5'-rGrUrArArGrArCrArCrGrArCrUrUrArUrCrGrCTT-3' (SEQ ID NO: 4)
The letter "r" preceding a nucleotide indicates that nucleotide is a ribonucleotide.

The annealed oligomers containing ColE1 sequence are referred to as siRNA-ori.

B. Delivery of target DNA and siRNA to liver cells in mice

Plasmid pMIR48 (10μg), containing the luc+ coding region (Promega Corp.) and a chimeric intron downstream of the cytomegalovirus major immediate-early enhancer/promoter, is mixed with 0.5 or 5μg of siRNA-luc+ and diluted in 1-3 mls Ringer's solution (147mM NaCl, 4mM KCl, 1.13mM $CaCl_2$) and injected in the tail vein over 7-120 seconds.

C. Assay of Luc+ activity and assessment of siRNA induction of RNAi

One day after injection, the livers are harvested and homogenized in lysis buffer (0.1% Triton X-100, 0.1M K-phosphate, 1 mM DTT, pH 7.8). Insoluble material is cleared by centrifugation. 10 μl of the cellular extract or extract diluted 10x is analyzed for luciferase activity using the Enhanced Luciferase Assay kit (Mirus).

Co-injection of 10μg of pMIR48 and 0.5μg of siRNA - luc+ results in 69% inhibition of Luc+ activity as compared to injection of 10g of pMIR48 alone. Co-injection of 5μg of siRNA- luc+ with 10μg of pMIR48 results in 93% inhibition of Luc+activity.

EXAMPLE 2

Inhibition of Luciferase expression by siRNA is gene specific in liver *in vivo*.

and veins to block both outflow and inflow of the blood to the leg. An efflux enhancer solution (e.g., 0.5 mg papaverine in 3 ml saline) is injected into the external iliac artery though a 25 - 27g needle, followed by the plasmid DNA and siRNA containing solution (in 10 ml saline) 1-10 minutes later. The solution is injected in approximately 10 seconds. The microvessel clips are removed 2 minutes after the injection and bleeding controlled with pressure and gel foam. The abdominal muscles and skin are closed with 4-0 dexon suture. Each procedure takes approximately 15 minutes to perform.

Four days after injection, rats were sacrificed and the quadricep and gastrocnemius muscles were harvested and homogenized as described in Example 1. Luc+ and Renilla Luc activities were assayed using the Dual Luciferase Reporter Assay System (Promega). Ratios of Luc+ to Renilla Luc were normalized to the siRNA-ori control. siRNA-Luc+ inhibited Luc+ expression in qaudriceps and gastrocnemius by 85% and 92%, respectively, compared to the control siRNA-ori.

EXAMPLE 10

RNAi of SEAP reporter gene expression using siRNA in vivo.

Single-stranded, SEAP-specific sense and antisense RNA oligomers with overhanging 3' deoxynucleotides are prepared and purified by PAGE. The two oligomers, 40μM each, are annealed in 250μl of buffer containing 50mM Tris-HCl, pH 8.0 and 100mM NaCl, by heating to 94^0C for 2 minutes, cooling to 90^0C for 1 minute, then cooling to 20^0C at a rate of 1^0C per minute. The resulting siRNA is stored at -20^0C prior to use.

The sense oligomer with identity to the SEAP reporter gene has the sequence:
5'-rArGrGrGrCrArArCrUrUrCrCrArGrArCrCrArUTT-3' (SEQ ID NO: 5)
and corresponds to positions 362-380 of the SEAP reading frame in the sense direction. The letter "r" preceding a nucleotide indicates that nucleotide is a ribonucleotide.

The antisense oligomer with identity to the SEAP reporter gene has the sequence:
5'-rArUrGrGrUrCrUrGrGrArArGrUrUrGrCrCrCrUTT-3' (SEQ ID NO: 6)

Single-stranded, cytosolic alanine aminotrasferase-specific sense and antisense RNA oligomers with overhanging 3' deoxynucleotides are prepared and purified by PAGE. The two oligomers, 40μM each, are annealed in 250μl of buffer containing 50mM Tris-HCl, pH 8.0 and 100mM NaCl, by heating to 94^0C for 2 minutes, cooling to 90^0C for 1 minute, then cooling to 20^0C at a rate of 1^0C per minute. The resulting siRNA is stored at -20^0C prior to use.

The sense oligomer with identity to the endogenous mouse and rat gene encoding cytosolic alanine aminotransferase has the sequence:
5'-rCrArCrUrCrArGrUrCrUrCrUrArArGrGrGrCrUTT-3' (SEQ ID NO: 7)
and corresponds to positions 928-946 of the cytosolic alanine aminotransferase reading frame in the sense direction. The letter "r" preceding a nucleotide indicates that nucleotide is a ribonucleotide.

The sense oligomer with identity to the endogenous mouse and rat gene encoding cytosolic alanine aminotransferase has the sequence:
5'-rArGrCrCrCrUrUrArGrArGrArCrUrGrArGrUrGTT-3' (SEQ ID NO: 8)
and corresponds to positions 928-946 of the cytosolic alanine aminotransferase reading frame in the antisense direction. The letter "r" preceding a nucleotide indicates that nucleotide is a ribonucleotide.

The annealed oligomers containing cytosolic alanine aminotransferase coding sequence are referred to as siRNA-ALT

Mice are injected in the tail vein over 7-120 seconds.with 40 μg of siRNA-ALT diluted in 1-3 mls Ringer's solution (147mM NaCl, 4mM KCl, 1.13mM $CaCl_2$). Control mice were injected with Ringer's solution without siRNA. Two days after injection, the livers were harvested and homogenized in 0.25 M sucrose. ALT activity was assayed using the Sigma diagnostics INFINITY ALT reagent according to the manufacturers instructions. Total protein was determined using the BioRad Protein Assay. Mice injected with 40 μg of siRNA-ALT had a 32% average decrease in ALT specific activity compared to that of mice injected with Ringer's solution alone.

Version showing changes made:

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